Noradrenergic and dopaminergic therapy in Parkinson's disease

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Chapter II

Effects of L-threo-DOPS on Central Noradrenaline Metabolism

2.1 Introduction
2.2 Materials and Methods
2.2.1 Animals
2.2.2 Study design
2.2.3 Microdialysis probe preparation
2.2.4 Microdialysis probe implantation
2.2.5 Dialysis procedure
2.2.6 Dialysate analysis
2.2.7 Analysis of glucose
2.2.8 Histological analysis
2.2.9 Drugs and chemicals
2.2.10 Data analysis and statistical analysis
2.3 Results
2.3.1 Biochemical effects of DOPS without DCI
2.3.2 Biochemical effects of DOPS with DCI
2.3.3 Effect of MHPG injection
2.3.4 Physiological and behavioural effects of DOPS without DCI
2.3.5 Effects of DOPS on plasma, urinary and dialysate glucose
2.3.6 Effects of DOPS on lung tissue
2.4 Discussion

2.1 INTRODUCTION

To be effective in alleviating central noradrenaline (NA) deficits in Parkinson's disease (PD), L-threo-DOPS (DOPS) should pass the blood-brain barrier (BBB), followed by efficient decarboxylation to NA. Besides, dopaminergic neurotransmission should not be impaired by DOPS. In this Chapter, the central decarboxylation of DOPS to NA is addressed. The influence of DOPS on central dopaminergic function will be studied in Chapter III.

DOPS was administered to rats equipped with a microdialysis probe implanted in the frontoparietal cortex motor area (FrPM). The FrPM is a projection field of the noradrenergic locus coeruleus (LC). Microdialysis in the FrPM of the rat allows to assess directly the influence of peripherally administered DOPS on central NA metabolism. An increase in the concentration of NA in the brain extracellular fluid (ECF) suggests that DOPS passes the BBB, and that DOPS is subsequently decarboxylated in the FrPM.

A potential limitation of the central efficacy of DOPS may be its peripheral decarboxylation to NA. Therefore, an experiment was performed with benserazide (BZ), a peripheral decarboxylase inhibitor (DCI).

The effects of DOPS on plasma and urinary glucose concentrations were measured to unravel the origin of DOPS-induced polydipsia, because in course of the experiment, animals treated with DOPS developed polydipsia and severe respiratory problems. Post mortem inspection of lung tissue clarified some of the respiratory problems.

To estimate the very low concentration of NA in the extracellular fluid under basal conditions, together with the several fold higher concentration of its metabolite MHPG in a single analytical run, a selective and highly sensitive HPLC analysis method was used.

Verhagen-Kamerbeek et al., 1991. In vivo assessment of the efficacy of L-threo-DOPS in producing extracellular noradrenaline in the CNS of the rat. In: Monitoring Molecules in Neuroscience, 373-376.
Verhagen-Kamerbeek et al., 1993. In vivo microdialysis studies on the role of L-threo-DOPS in the treatment of Parkinson's disease. In: Norepinephrine deficiency and its treatment with L-threo-DOPS in Parkinson's disease and the related disorders, 49-58.

2.2 MATERIALS AND METHODS

2.2.1 Animals

Male adult albino Wistar rats (CDL, Groningen, The Netherlands) were used. For adaptation to the experimental conditions, animals were housed individually in perspex cages measuring 40x40x40 cm under 12 h/12 h light/dark conditions (7.00 h lights on/19.00 h lights off) at 21°C and 40-50% humidity, at least one week before the experiment. The animals had free access to standard food and drinking water. Isolation of the animals was not complete, since neighbours were visible through the perspex, and animals smelled each other through the open roof of the cages. Animals were handled daily during control of bodyweight. Only rats with normal bodyweight gains (with reference to the growth curve of healthy Wistar rats under the same conditions) during the week prior to the experiment, and normal recovery from surgery without significant loss of bodyweight (> 10% of presurgery bodyweight) were used in the experiments.

2.2.2 Study design

Animals were divided into six groups. Animals in Groups I, II, III, IV and V were equipped with a microdialysis probe in the FrPM. Group I served as control, receiving a single injection of saline (n=4). As an inventory on the pharmacologically effective dose of DOPS, 10 animals in Group II received different doses of DOPS (50, 100, 200 and 400 mg/kg, n=2 or 3 per dose). Group III was treated with 100 mg/kg DOPS without a peripheral DCI (n=4). Group IV was treated with 2 mg/kg BZ 30 min prior to injection of 100 mg/kg DOPS (n=4).

Animals in Groups V and VI were implanted with an intrajugular cannula according to the method of Steffens (1969) to allow repetitive sampling of blood and intravenous drug administration. Group V was infused with 100 μl of saline containing 400 μg/kg MHPG (n=4). Group VI (n=17) was treated with 10, 50 or 100 mg/kg DOPS. Before injection of DOPS one sample of blood (100 μl) was drawn and kept on ice untill further handling. After injection of DOPS, blood (100 μl) was drawn every 30 min for 120 min. Two animals of Group VI were also implanted with a dialysate probe in the FrPM and in the striatum. After treatment with a single dose of 10 or 100 mg/kg DOPS, microdialysates were collected from both the FrPM and striatum. Blood was drawn before injection of drug and every 30 min after injection. The interior of the animals in Group VI was examined post mortem. The location of the intrajugular cannula was verified, lungs were excized and photographed, and a sample of urine was taken from the bladder by needle expiration.

2.2.3 Microdialysis probe preparation

Microdialysis probes were made according to the procedure described in detail elsewhere (Imperato and DiChiara, 1984). One ending of a piece of semi-permeable microdialysis fibre supported by a tungsten wire (TW5-3, 125 mm, Portanje Electronica BV, Bunnik, The Netherlands) was glued inside a suitable hypodermic needle (22G and 23G for preparation of striatal and cortical probes, respectively). The areas of the probe not exposed for dialysis were marked and covered with silicone glue. The cortical probe (C-DAK 201-810D 90SCE cellulose fibre, 340 μm OD, MW cut-off 10,000 Da, CD-Medical, Roden, The Netherlands) used in this study had a length of 8 mm of the fibre exposed to brain tissue. The striatal probe (polyacrylonitrile/sodium methalyl sulphonate copolymer fibre, 500 μm OD, MW cut-off 5,000 Da, HOSPAL, Italy) had 4 mm of fibre

exposed to each striatum. Before implantation, the free end of the fibre was glued to the tungsten wire. In Fig. 2-1 transversal probes designed for dialysis in both the right and left striatum, and for the FrPM are presented.

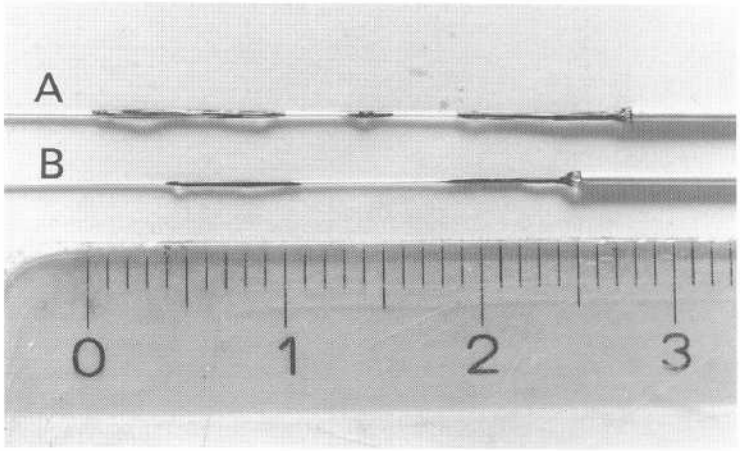

Figure 2-1. *Photograph of transversal microdialysis probes for implantation in (A) the striatum, and (B) in the FrPM of the rat. One end of the fibre, which is supported by a tungsten wire, is glued inside a hypodermic needle to allow clamping into the stereotactic instrument and to connect the dialysis tubing. The other end of the probe is inserted into the brain. The parts of the probe passing through brain structures that are not of interest for biochemical monitoring are covered with silicon glue. The blank parts of the probe allow free interchange of compounds between the perfusion liquid and the interstitial fluid of the brain.*

2.2.4 Microdialysis probe implantation

On the first day (day 0) of the experiment, a transversal microdialysis cannula was implanted in the FrPM under sodium pentobarbitone (60 mg/kg, i.p.) anaesthesia according to the procedure for implantation in the rat striatum described in detail elsewhere (Imperato and DiChiara, 1984). This procedure was adapted for implantation in the FrPM. Animals weighed 230-260 g at the time of operation. During surgery physiological conditions (respiration, temperature) were controlled. Anaesthesia was maintained by additional injections of the anaesthetic. The one end of a previously prepared microdialysis probe covered with the needle was clamped horizontally in a holder on the right bar of a David Kopf stereotaxic instrument. Animals were mounted in the stereotaxic frame with the incisor bar set at 2.5 mm below the interaural line. The skull was exposed and two holes (approximately 2 mm diameter) were drilled at either side of the skull at the level of the FrPM. The dura was carefully removed with a hypodermic needle. Coordinates for stereotaxic implantation of the dialysis fibre were modified from the stereotaxic atlas of Paxinos and Watson (1986). The probe was inserted into the FrPM at coordinates +0.5 mm A with reference to bregma, and -2.0 mm V from the surface of the skull. In case of implantation in the striatum (Chapter III) the striatal probe was inserted at coordinates +1.5 mm A, and -5.5 mm V. For implantation of a

probe in both the FrPM and in the striatum (Chapter III), first the striatal probe was inserted followed by placement of the cortical probe. After implantation, the glued part of the probe(s) was cut off, and the free fibre ending was glued inside a suitable piece of hypodermic needle. After the tungsten wire was removed, both ends of the probe(s) were secured on the skull with dental cement. The wound was closed with a few stitches, muscle and skin being sutured separately.

After surgery the animal returned to its home cage to recover. To be sure that perfusates were not contaminated with plasma NA after surgery, a 48 h period was taken for closure of occasional BBB disrupture, and physical recovery of the animal.

2.2.5 Dialysis procedure

Experiments were conducted at day 2 after surgery in awake freely moving animals during the light period in the home cage. The experiment was started with the connection of the probe to the microperfusion pump (Harvard Classic Mechanical Microliter Syringe Pump, Plato BV, Diemen, The Netherlands), which delivered a Ringer solution containing Na^+ 147 mM, K^+ 4 mM, Ca^{2+} 2.3 mM and Cl^- 155 mM (pH 6.0) through the cannula at a rate of 3.05 μl/min. Perfusates were collected manually every 30 min in chilled (4°C) vials containing 10 μl 0.01 N HCl to prevent auto-oxidation. Usually 3 or 4 perfusates were collected under control conditions prior to drug administration. Dialysates were stored at -70°C until assessment of the amount of drug, neurotransmitters and metabolites.

2.2.6 Dialysate analysis

An analytical procedure was developed for the simultaneous determination of the concentration of NA, MHPG, DA, DOPAC, HVA, and 5-HIAA in cortical and striatal dialysates in one chromatographic run. Without sample clean-up dialysates were analyzed off-line, which enabled splitting of the dialysate for determination of recovered native substances, and the concentration of the drug in a single fraction. An isocratic HPLC-pump (LKB 2150, Pharmacia Nederland BV, Woerden, The Netherlands) was coupled to an amperometric electrochemical detector (ANTEC Leyden, Leiden, The Netherlands) operating at an oxidation potential of 700 mV versus an Ag/AgCl reference electrode. Compounds were separated by ion-pair chromatography. A reversed phase Velosep C18 column (Brownlee Labs, Santa Clara, CA, USA) 100 x 3.2 mm, 3 μm particle size was used as the analytical column. No guard column was used. The mobile phase contained 0.1 M acetic acid, 1 mM sodium octanesulphonic acid, 0.4 mM EDTA, 4% methanol v/v. This mixture was adjusted to pH 4.55 with 2N NaOH. The mobile phase was constantly degassed by a stream of He. Recycling of the mobile phase created a constant background signal and reproducible chromatographic conditions. The flow rate was 0.7 ml/min. The detection limit was 2 pg/50 μl injection volume. Separation, sensitivity and retention times of the compounds are presented in Fig. 2-2. The concentrations of monoamines and metabolites were not corrected for the recoveries of the probes for these compounds.

2.2.7 Analysis of glucose

Plasma and dialysate glucose were measured with a standard enzymatic colorimetric assay. The glucose level in urine was determined using a semi-quantitative indicator stick with sensitivity range 100-1000 g/l.

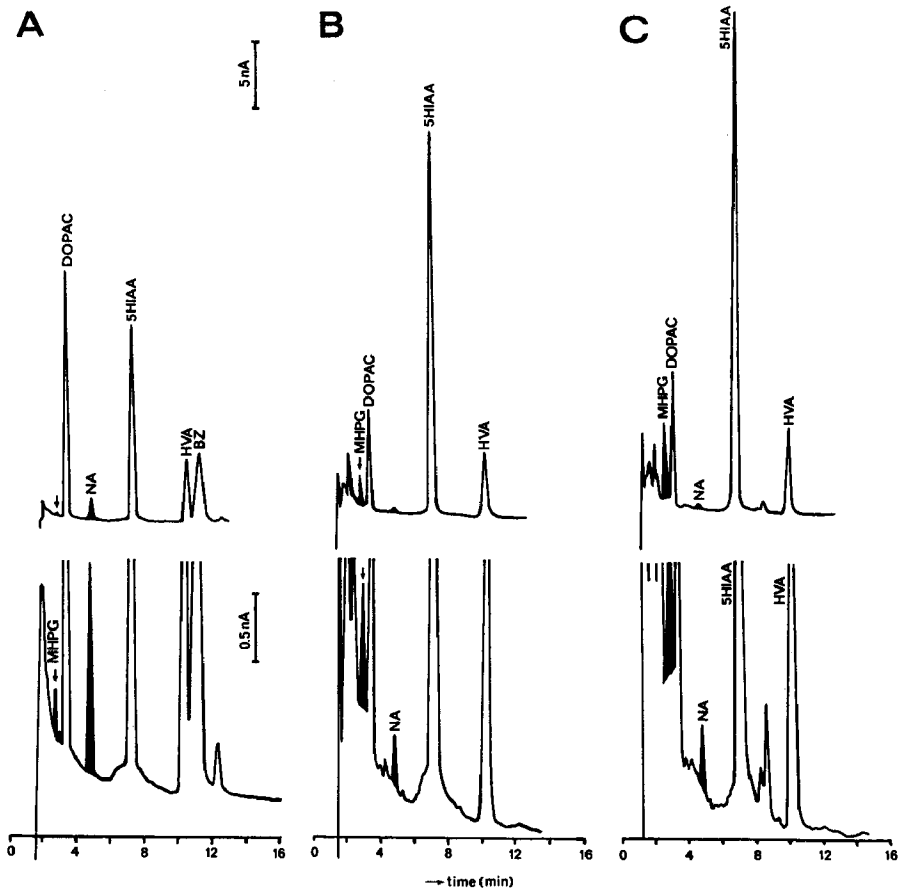

Figure 2-2. *Chromatograms of 50 µl of (A) a standard solution containing 50 pg MHPG and NA, and 500 pg DOPAC, 5HIAA, HVA, and BZ. (B) FrPM dialysate under baseline conditions. (C) FrPM dialysate collected 90 min after injection of 200 mg/kg DOPS.*

2.2.8 Histological analysis

After the experiment animals were killed under deep sodium pentobarbitone anaesthesia by transcardial perfusion-fixation with 4% paraformaldehyde in phosphate buffered saline (PBS). To visualize the track of the dialysis probe, brains were removed from the skull, post-fixed in the same fixative, dehydrated in a 25% sucrose solution in PBS, and sectioned on a cryostat. Sections (30 µm) were mounted on glass slides and routinely stained with cresyl violet. The stained sections were evaluated microscopically to check correct placement of the probe and the extent of tissue damage.

2.2.9 Drugs and chemicals

All drugs were dissolved in saline except DOPS which was suspended in 0.5 % methyl-

cellulose in saline. The drug solutions/suspensions were freshly prepared on the day of the experiment. Drugs were administered by i.p. injection. L-threo-DOPS was a gift from Sumitomo Pharmaceutical Company Ltd., Osaka, Japan. Carbidopa and benserazide were purchased from Bufa Chemie, Castricum, The Netherlands. MHPG-hemipiperazine was purchased from Sigma, St Louis, MO, USA. All chemicals were reagent or HPLC grade.

2.2.10 Data analysis and statistical analysis

Concentrations in dialysate are presented in pg/50 μl, uncorrected for probe recoveries. The average concentration of a compound in the 3 or 4 dialysates collected before drug administration was normalized to 100%. The SEMs of the basal values were less than 5% of the means. After administration of drug, concentrations were expressed as percentage of the basal value. The effect of treatment was assessed with the paired Student's *t* test compared with basal pre-injection conditions. Differences between groups were analyzed with the nonparametric Mann-Whitney U test (Siegel, 1956). Correlations (linear regression) and differences were considered statistically significant when $p < 0.05$.

2.3 RESULTS

2.3.1 Biochemical effects of DOPS without DCI

The baseline concentrations of NA, MHPG, DOPAC, HVA and 5HIAA in FrPM dialysate are summarized in Table 2-1.

Table 2-1. *Baseline concentrations of NA and monoamine metabolites in rat FrPM dialysate.*

constituent	concentration[a]
NA	5.0± 2.3 (23)
MHPG	113 ± 44 (28)
DOPAC	144 ± 90 (25)
HVA	334 ±192 (26)
5HIAA	863 ±256 (24)

[a] *Mean ± SD concentration expressed in pg/50 µl dialysate, uncorrected for probe recovery. Number of animals in parentheses.*

Injection of saline in Group I had no effect on the concentration of NA, but caused a slight transient increase of MHPG in FrPM dialysate. In Group II the concentrations of NA and MHPG were quantified as possible indices for effective central decarboxylation of DOPS. The maximal dose in this study was 400 mg/kg of the pure L-isomer, which is comparable to a dose of 800 mg/kg administered by others using racemic DL-DOPS. NA remained within the baseline range in animals treated with 50 and 100 mg/kg DOPS. NA increased in FrPM dialysates of animals treated with 200 and 400 mg/kg DOPS (Fig. 2-3A). Notably, a dose of 100, 200 and 400 mg/kg resulted in animal behaviour suggestive of toxic rather than pharmacological effects of DOPS. These behavioural changes and seemingly adverse effects are described in Section 2.3.4. A rise in central NA was observed only in animals suffering from these adverse effects.

Immediately after administration of DOPS at any dose the concentration of MHPG in dialysate was elevated markedly in a dose-dependent fashion (Fig. 2-3B). There was a poor linear relationship between the dose of DOPS and NA ($r=0.87$, $p<0.05$), whereas the concentration of MHPG showed a good linear relationship with the dose of DOPS ($r=0.96$, $p<0.001$).

In Group III treated with 100 mg/kg DOPS the concentration of NA in dialysate was not changed significantly compared to baseline NA. MHPG, but also the dopamine (DA) metabolites DOPAC and HVA, as well as 5HIAA increased. These data are presented in Fig. 2-4.

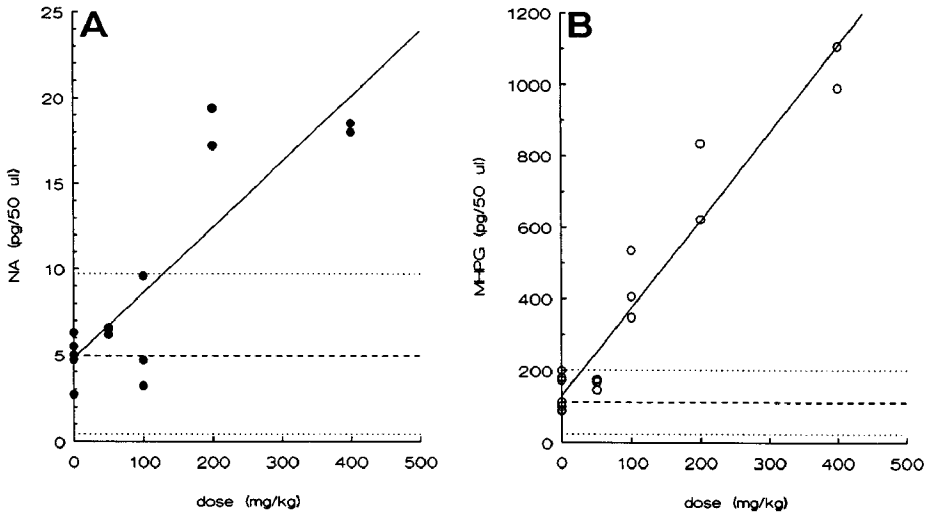

Figure 2-3. *Study in 10 animals treated with different doses of DOPS (Group II). (A) Plot of the maximal concentration of NA as function of the dose of DOPS (●, $r=0.87$, regression line $Y=4.76 + 0.04X$, $p<0.05$, $n=20$). (B) Plot of the maximal concentration of MHPG as function of the dose of DOPS (○, $r=0.96$, regression line $Y=129 + 2.45X$, $p<0.001$, $n=20$). Each marker represents a single experiment. The amount recovered in dialysate was not corrected for probe recovery, and expressed as pg/50 µl. Mean basal concentration (----)±2SD (·····).*

2.3.2 Biochemical effects of DOPS with DCI

Since 100 mg/kg DOPS did not cause a significant rise of NA in FrPM dialysate, animals in Group IV were treated with a combination of DOPS and BZ to enhance the central availability of DOPS. Animals were treated with 2 mg/kg BZ 30 minutes before the dose of DOPS. An oral dose of 10 mg/kg BZ efficiently inhibits peripheral decarboxylase activity, but does not affect central decarboxylase activity as measured in the rat striatum (Da Prada et al., 1984). Peripheral inhibition of decarboxylation by BZ resulted in no significant increase in the extracellular NA concentration in the FrPM. BZ prevented the increase in MHPG and 5HIAA that was induced by treatment with DOPS alone (Figs. 2-4A and 2-4B). In contrast, the rise in the concentration of DOPAC after DOPS alone was not influenced by BZ pretreatment (Fig. 2-4C). Co-administration of BZ and DOPS

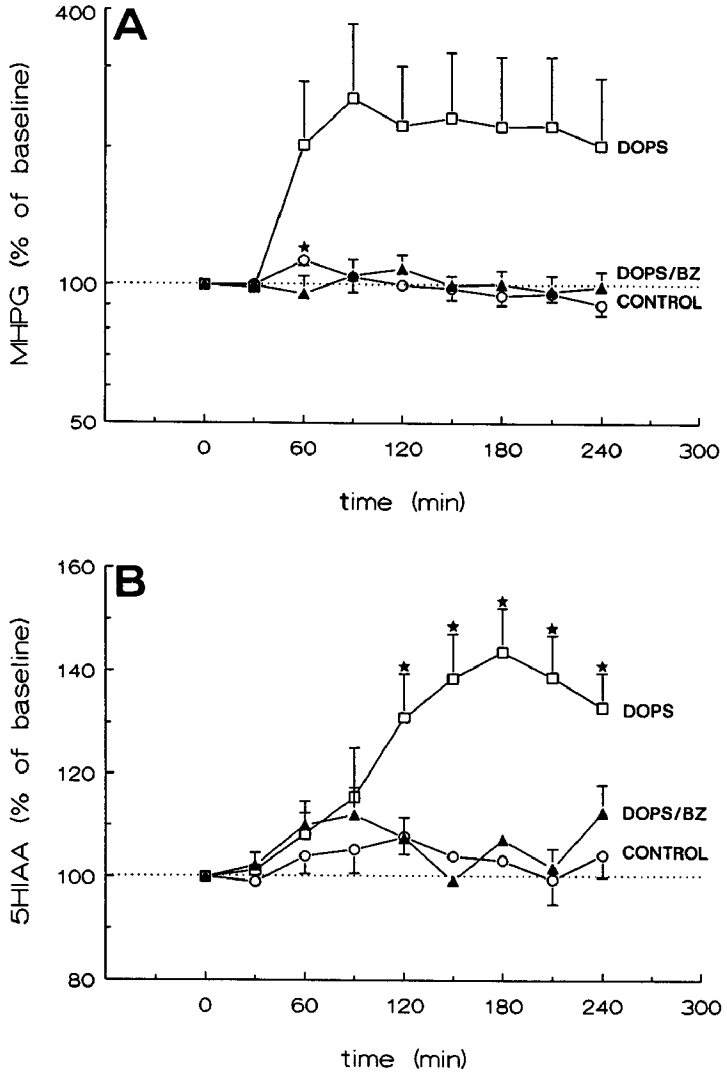

Figure 2-4. *Effects of injection of saline (○, Group I), injection of 100 mg/kg DOPS (□, Group III) and 100 mg/kg DOPS + 2 mg/kg BZ (▲, Group IV) on the concentrations of (A) MHPG, (B) 5HIAA, (C) DOPAC, and (D) HVA in FrPM dialysate. The average amount recovered under baseline conditions was normalized to 100%. The amount in dialysate after administration of drug is expressed as percentage of the basal concentration. Each value represents the mean (±SEM) of 4 determinations.* $^{*}p<0.05$, $^{**}p<0.01$ *(paired Student's t test).*

increased HVA to concentrations that differed significantly from those after treatment with DOPS alone ($p<0.01$, Mann-Whitney U test) (Fig. 2-4D).

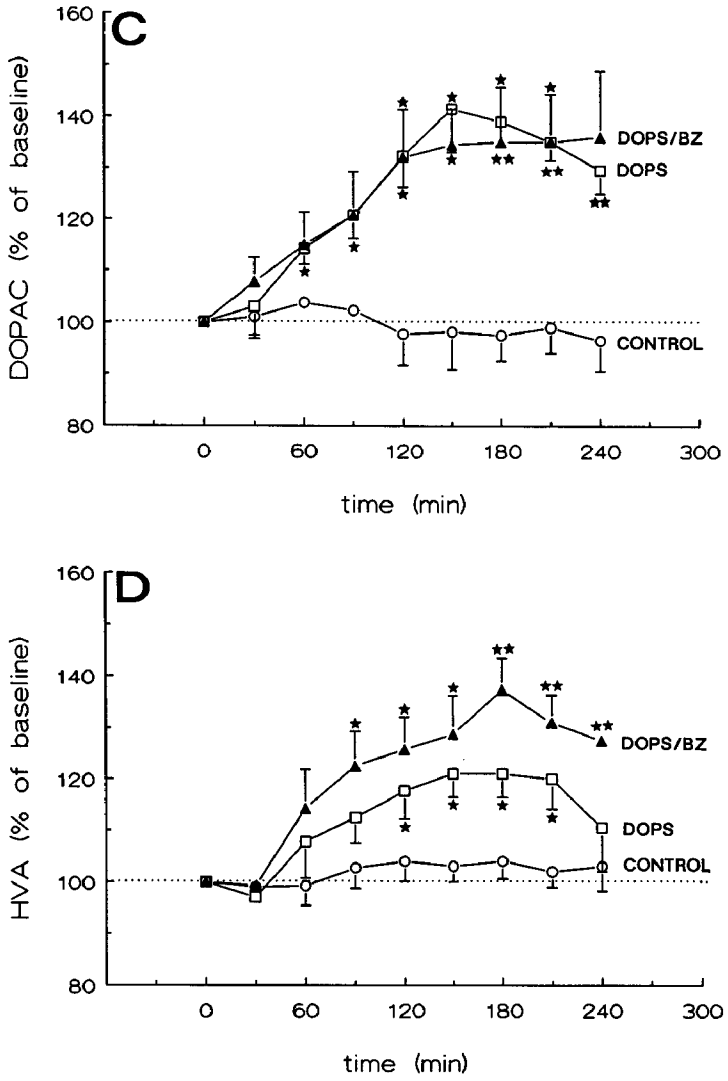

Figure 2-4. *Legend on opposite page.*

2.3.3 Effect of MHPG injection

Co-administration of BZ and DOPS in Group IV prevented the increase in dialysate MHPG which was induced by DOPS alone. This suggested that the central level of MHPG reflected both central NA-turnover and peripheral DOPS metabolism to NA and MHPG. In Group V intravenous injection of 400 μg/kg MHPG caused an immediate significant rise of FrPM extracellular MHPG without altering the concentration of NA

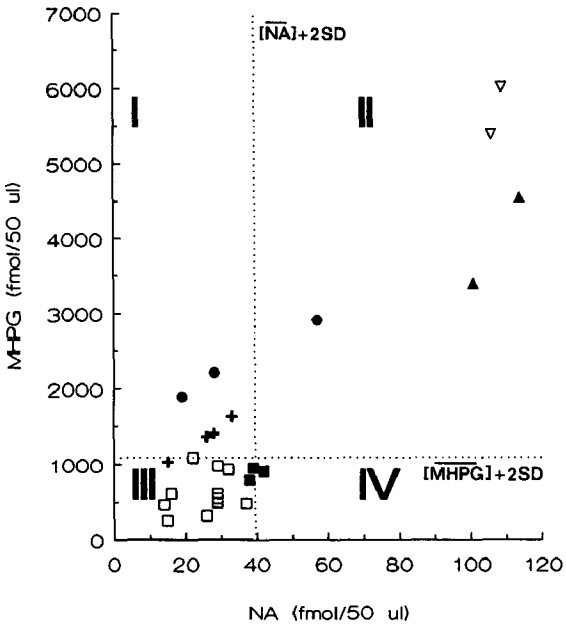

Figure 2-5. *Relationship between the concentrations of NA and MHPG in FrPM dialysate under baseline conditions (□, n=14), and the relationship between the maximum concentration of NA and MHPG after intravenous infusion of 400 μg/kg MHPG (✚, n=4). The relationship between the maximum dialysate concentrations of NA and MHPG after injection of different doses of DOPS studied in Group II is also illustrated; 50 mg/kg (■, n=3), 100 mg/kg (●, n=3), 200 mg/kg (▲, n=2), and 400 mg/kg (▽, n=2). Each marker represents a single experiment. The amount recovered in dialysate was not corrected for probe recovery, and expressed as fmol/50 μl. Dotted lines indicate the mean basal concentration + 2SD of NA and MHPG.*

Note the clear deviation from the basal MHPG concentration (quadrant III) after peripheral infusion of MHPG (quadrant I) without a significant change in the basal NA concentration. Injection of 50 mg/kg DOPS does not affect the relationship between NA and MHPG significantly. 100 mg/kg increases MHPG without a significant change in NA in 2 out of 3 animals (quadrant I). After injection of 200 and 400 mg/kg both NA and MHPG concentrations in dialysate are elevated (quadrant II).

(Fig. 2-5). MHPG reached its peak concentration 1 h after injection and returned to baseline within 2.5 h. In contrast, in Groups II and III treated with DOPS without DCI, MHPG remained at its maximal concentration for several hours (Fig. 2-4A).

2.3.4 Physiological and behavioural effects of DOPS without DCI

DOPS given in doses ≥100 mg/kg without DCI evoked a complex pattern of physiological and behavioural changes, eventually resulting in death of the animal within 10 h after administration. The adverse effects augmented with increasing doses of DOPS. The effects of DOPS included tachycardia, piloerection, exophtalmia, photophobia, and peripheral vasoconstriction resulting in a pale tail, nose and ears, and cold extremities. Inspiration was superficial and expiration was troublesome resulting in shortness of breath

and suffocation. Foamy mucus contaminated with blood was secreted from the nose. The tail was lifted in the air in a perpendicular direction. Animals were lying flat on the ground with their hindlimbs seemingly paralyzed. Locomotion was characterized by crawling movements with the forelimbs. Movements were directed towards the water flask. After the animals had reached the flask, they drank uninterruptedly. This complex behavioural pattern was prevented by injection of 10 mg/kg BZ or 20 mg/kg carbidopa 30 min prior to or within 30 min after administration of DOPS.

2.3.5 Effects of DOPS on plasma, urinary and dialysate glucose

DOPS rapidly increased the plasma concentration of glucose in a dose-dependent fashion. Table 2-2 summarizes the effects of DOPS on plasma and urinary glucose of animals in Group VI. The concentration of plasma glucose at 30 min was used to compare the effect on plasma glucose between different doses of DOPS, because at this time point it was possible to collect blood from the majority of the animals studied. The maximal concentrations of plasma glucose were: 10 mg/kg DOPS, 10 mmol/l at 90 min after injection; 50 mg/kg DOPS, 20.7 mmol/l at 60 min after injection; 100 mg/kg DOPS, 12.5 mmol/l at 30 min after injection of (all cannulas blocked between 30-60 min post injection).

Table 2-2. *Effect of DOPS on the plasma and urinary glucose concentration of the rat.*

dose DOPS	plasma glucose[a]		glycosuria
	baseline	effect[b]	
10 mg/kg (5)	7.3±0.4 (3)	9.0±0.2 (2)[c]	20%[e]
50 mg/kg (8)	6.7±1.2 (6)	13.0±1.8 (6)[d]	88%
100 mg/kg (4)	5.6±0.6 (3)	12.5 (1)	100%

[a] *Mean ± SD plasma glucose concentration expressed in mmol/l. Number of animals in parentheses.*
[b] *Concentration measured at 30 min after injection of DOPS.*
[c] *Due to obstruction of the intrajugular cannula in course of the experiment it was not possible to monitor the plasma glucose concentration in all animals for the entire period of 4 h after injection.*
[d] *$P<0.001$ compared to basal plasma glucose levels (paired Student's t test, $n=6$).*
[e] *Percentage of animals studied with positive glucose test in urine at 4 h after injection of DOPS.*

There was glycosuria in 1 out of 5 animals treated with 10 mg/kg DOPS, whereas 7 out of 8 animals developed glycosuria after treatment with 50 mg/kg DOPS. There was glycosuria in all 4 animals treated with 100 mg/kg (Table 2-2).

In animals treated with BZ prior to DOPS, plasma glucose was not determined, but these animals did not display polydipsia, nor glycosuria.

The concentration of glucose in dialysate was measured in two rats equipped with a transversal cortical and striatal dialysis probe. One animal was treated with 10 mg/kg DOPS, the other with 100 mg/kg DOPS. Although the experiment was performed in only two animals and with different doses of DOPS, the basal glucose concentrations in cortical and striatal dialysates were elevated undoubtedly (Table 2-3). Following DOPS

Table 2-3. *Effect of DOPS on the glucose concentration in cortical and striatal dialysate.*

dose DOPS	cortical glucose[a]		striatal glucose	
	baseline	effect[b]	baseline	effect
10 mg/kg (1)[c]	0.090	0.155	0.290	0.380
100 mg/kg (1)	0.115	0.175	0.140	0.710

[a] *Dialysate glucose concentration expressed in mmol/l.*
[b] *Effect measured at 150 min after injection of DOPS.*
[c] *Number of animals in parentheses.*

injection dialysate glucose levels increased rapidly, and remained at concentrations above baseline values for at least 2 h. The rise was more pronounced after 100 mg/kg DOPS. The time profile of the glucose concentration in dialysate paralleled the profile in plasma.

2.3.6 Effects of DOPS on lung tissue

Since respiratory problems were the most prominent among the adverse events described in Section 2.3.4, lung tissue of animals treated with DOPS was examined for aberrations from normal rat lung tissue. Upon opening of the thorax, the lungs showed pulmonary edema with congestion of the alveolar tubes. Moreover, many hemorrhages were found. None of these pathological signs were developed after treatment with BZ prior to DOPS. Figure 2-6 shows the lungs of an animal treated with 100 mg/kg DOPS, and the preventive effect of prior treatment with BZ in another animal treated with the same dose of DOPS.

2.4 DISCUSSION

There is general agreement on the conversion of DOPS to NA in peripheral tissues, like the rat heart and kidney (Ohmura et al., 1978; Araki et al., 1981; Reches et al., 1985; Soares da Silva, 1993). In humans DOPS increases plasma NA (Suzuki et al., 1985; Man in 't Veld et al., 1987), and secretion of NA in the urine (Suzuki et al., 1980). However, reports regarding the central conversion of DOPS to NA are divergent. Some authors claim that DOPS was decarboxylated in the CNS since they found an increase in the concentration of NA in human CSF (Tohgi et al., 1990; 1993), or in brain tissue of rats and mice (Semba and Takahashi, 1985; Kato et al., 1986, 1987; Koshiyama et al., 1987). Others found that NA levels were inconsistently increased in human CSF (Suzuki et al., 1984) or that NA levels in rat and mouse brain tissue were unaffected by peripherally administered DOPS (Puig et al., 1974; Hirai et al., 1975; Reches et al., 1985; Mori et al., 1987; Gibson, 1988). It is conceivable that by examining the action of DOPS on whole brain homogenates, interpretation of its action specifically in brain tissue might be obscured by a contribution of peripherally formed vascular NA, or NA formed after conversion of DOPS by brain capillary AADC (Bartholini et al., 1971; Constantinidis and Geissbühler, 1971; Hardebo et al., 1979). We therefore explored the formation of NA in brain tissue by measuring the overflow of NA in the FrPM extracellular fluid of the rat by means of *in vivo* microdialysis. The FrPM is a major terminal domain of nor-

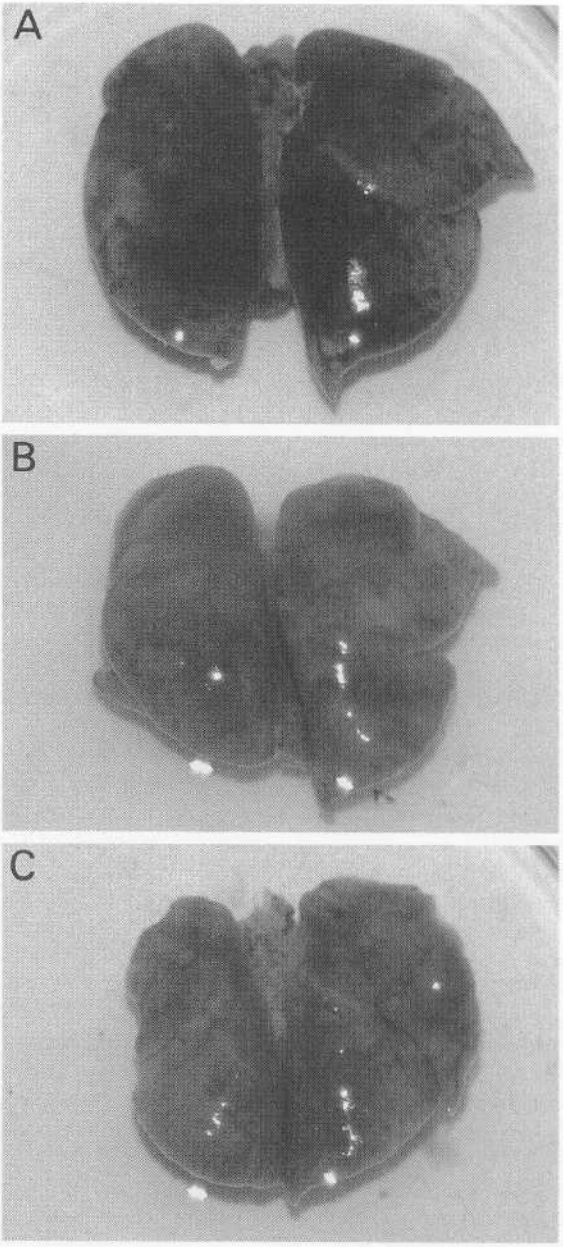

Figure 2-6. *(A) Typical example of the effect of 100 mg/kg DOPS on lung tissue excized 120 min after i.p. injection.* ***(B)*** *The lungs of a rat treated with 10 mg/kg BZ three days before treatment with 100 mg/kg DOPS.* ***(C)*** *The lungs of a control animal. Note the hemorrhages in the lungs of the rat treated with DOPS alone, and the apparent protective effect on lung tissue of BZ treatment prior to DOPS injection.*

adrenergic fibres originating in the LC, while degeneration of noradrenergic cell bodies in the LC is part of PD pathology (Chan-Palay and Asan, 1989).

In our experimental setting limitation of the influence of environmental stress was achieved by performing the experiments in the homecage placed in an environment with background noise to which the animals had been accustomed. Furthermore, animals were not restricted in their freedom of movement by the microdialysis tubing. The intraperitoneal injection of saline did not cause a significant change in extracellular FrPM NA, although MHPG was slightly elevated. Thus it was excluded that the effect of DOPS on central NA was due to injection stress. These precautionary measures were taken because of the known effects of environmental and sensory stress on NA release in the brain (Korf et al., 1973; review Glavin, 1985; Abercrombie et al., 1988; Kalén et al., 1988; Takita et al., 1992; Nakata et al., 1993), and that intense or chronic stress even reduces brain NA (Barchas and Freedman, 1963; Bliss and Zwanziger, 1966; Stone, 1973; Svensson, 1987; Adell et al., 1988).

Unambiguous interpretation of the central effects of DOPS on NA levels is difficult in terms of the net effect of DOPS on FrPM NA, since peripherally formed NA causes considerable peripheral metabolic shifts, including a rise in blood pressure which inhibits LC function and reduces NA metabolism in brain regions innervated by the LC (Svensson, 1987). At the doses used, non-physiological high concentrations of circulating NA were generated that induced a syndrom of catecholamine intoxication (Hoppe et al., 1949). Peripheral conversion of DOPS to NA may thus account for the lack of effect or a net inhibitory effect on central NA function. However, concomitant administration of BZ did not facilitate the formation of NA in the CNS. Attempts with co-administration of DOPS and 20 mg/kg desipramine, together with the α_2-adrenoceptor antagonist yohimbine (5 mg/kg, s.c.), failed to show a central production of NA by DOPS.

The concentration of free MHPG in CSF and brain tissue has been used as an index of the central NA formation by DOPS (Ogawa et al., 1984; Edwards and Rizk, 1981; Kato et al, 1987; Karai et al., 1987; Takubo and Kondo, 1988). Although brain and CSF levels of MHPG have been used as an index of central NA metabolism (Chase et al., 1973; DeMet and Halaris, 1979; Elsworth et al., 1982), it is necessary to correct these values for the contribution of plasma free MHPG, because free MHPG crosses the BBB (Jimerson et al., 1981; Kopin et al., 1983; Faull et al., 1990). In other words, tissue, CSF and ECF concentrations of MHPG do not reflect exclusively brain NA-turnover, since part of CSF MHPG is of peripheral origin (Wolfson and Escriva, 1976). Therefore, the rise in the concentration of MHPG in brain tissue homogenates or in CSF after DOPS is of limited value as an index of central NA formation. This conclusion is supported by the finding that after DOPS administration MHPG was elevated even in brain areas that are devoid of AADC (Gibson, 1988), and that our intravenous infusion of free MHPG resulted in an increase in dialysate MHPG. With respect to the interpretation of the effect of DOPS on central NA turnover, an increase in brain MHPG does not suffice as evidence of central NA formation. Our findings are in accordance with those of others, who found that after 50 mg/kg DOPS p.o. brain MHPG was contaminated with peripherally formed MHPG (Keller et al., 1987).

The finding that 5HIAA, DOPAC and HVA were increased in FrPM dialysate after treatment with DOPS alone awaits an explanation, especially since BZ co-treatment prevented the increase in 5HIAA, but enhanced the effect on HVA. Thus, displacement of endogenous 5HT from nerve terminals by DOPS can be ruled out (Nishino et al., 1987). Presumably, due to the pressor response of DOPS (Araki et al., 1981; Morimoto et al.,

1990) LC activity is reduced (Svensson, 1987), which leads indireetly to a reduction of the inhibitory modulation of the LC on the activity of serotonergic raphe neurons (Fuxe et al., 1978). This results in activation of the serotonergic neurons in the raphe projection fields, which may be reflected in the concentration of 5HIAA in FrPM dialysate. Moreover, treatment with BZ prevented both the increase in 5HIAA and the severe pharmacodynamical events. It may be of interest, however, that DOPS with and without BZ promotes FrPM DA turnover. In this context, the discussion on the effect of DOPS on motor function in PD is still open, since a stimulatory action of DOPS on basal ganglia DA function may explain the beneficial effect of DOPS in the treatment of PD.

We found that systemic DOPS administration caused a rise in plasma and urinary glucose that was paralleled by a rise in dialysate glucose recovered from the FrPM and striatum. It is conceivable that the effect of DOPS on peripheral glucose metabolism was the result of a direct sympathetic effect on liver gluconeogenesis and liberation of glucose from liver or skeletal muscle glycogen mediated by NA rather than DOPS itself, because no glycosuria or rise in plasma glucose were measured when DOPS was combined with a DCI. Moreover, noradrenergic stimulation of α_2-adrenoceptors on the pancreatic ß-cells leads to inhibition of the release of insulin, and ß-adrenergic stimulation of glucagon secretion. Our finding of increased amounts of glucose excreted in the urine confirms the DOPS-induced hyperglycemia. The finding by others of a diuretic effect of DOPS, which also appeared sensitive to treatment with a DCI, is in good agreement with our findings on an elevation of plasma glucose (Katsube et al., 1986; Morimoto et al., 1990). Our problems with obstruction of the intrajugular cannula may be explained by non-neuronal stimulation of α_2-adrenoceptors promoting platelet aggregation, an effect which is induced at non-physiological concentrations of circulating catecholamines (Barthel and Markwardt, 1974; Haft et al., 1972).

Under physiological conditions, part of NA in the systemic circulation is eliminated by the lungs (Halbrügge et al., 1988). After administration of large doses of DOPS, however, peripheral formation of a non-physiological concentration of circulating NA together with the local metabolic activity of AADC in the lungs (Porter, 1973) resulted in pulmonary edema and congestion of the lungs. It appears also of importance that three days after a single 10 mg/kg BZ injection, BZ or its active metabolite was still capable to prevent peripheral decarboxylation adequately, as judged by the effect of DOPS on lung tissue. Finally, since catecholamines are also involved in peripheral aspects of temperature regulation, the panting respiration of the animals might be a way to increase loss of body heat (Ganong, 1977). In the study by Teelken and co-workers (1989) oral administration of 1500 mg/day DL-DOPS to a patient with PD produced excessive perspiration (unpublished observation), a phenomenon which has been described also in pheochromocytoma and diabetes mellitus (Prout and Wardle, 1969; Edmonds and Watkins, 1990). DOPS promoted perspiration in two cases of dopamine ß-hydroxylase (DßH) deficiency (Mathias et al., 1990). Moreover, in mice treated with FLA-63, a DßH inhibitor, the induced reduction in body temperature was restored by DOPS (Svensson, 1971).

In conclusion, *in vivo* microdialysis is a suitable technique to study the potency of DOPS as a central NA precursor. Because the NA level did not rise in the FrPM neither after DOPS, nor after DOPS/DCI administration, there is no experimental evidence to support decarboxylation of DOPS in the rat FrPM. MHPG appears not a suitable marker for the efficacy of DOPS as a NA precursor in the CNS in studies without DCI since combination with a DCI prevented the increase in central MHPG. Although DOPS

induced clear sympathomimetic effects, the overt signs of toxicity without central NA changes, warrant careful application in (aged) humans and parkinsonian patients. Our results do not exclude a role of central non-noradrenergic mechanisms in the action of DOPS, since DOPS elevated central DOPAC and HVA, suggesting that DOPS influences central DA turnover. The studies in Chapter III address the question whether DOPS exerts its therapeutic action in the treatment of akinetic symptoms in Parkinson's disease via potentiation of basal ganglia DA release.